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Potato meristem culture and virus X

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POTATO MERISTEM CULTURE AND VIRUS X

by

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Head of Major Department

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Dean of Graduate College

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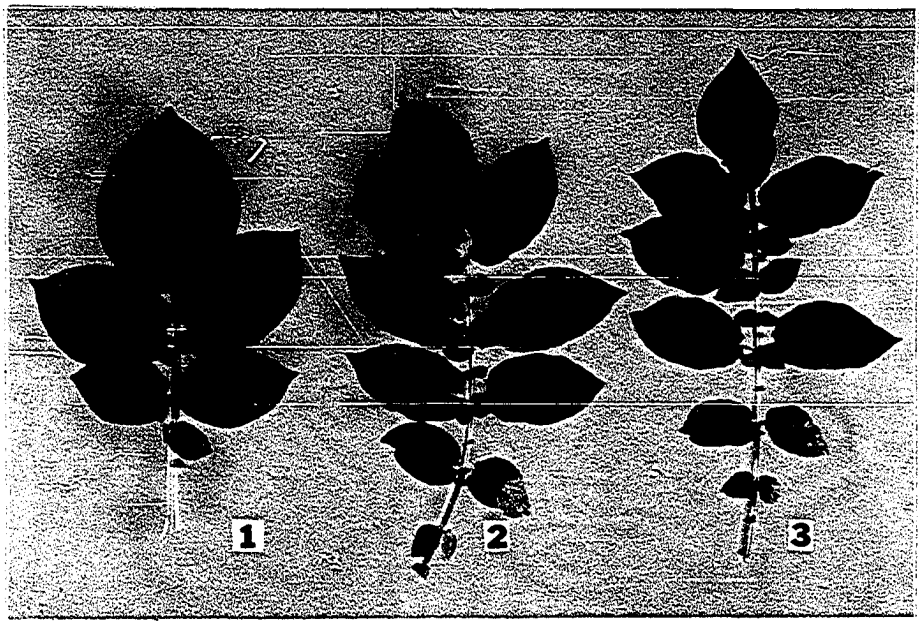
INTRODUCTION

Virus X disease, now known throughout the potato growing regions of the world, was first recognized when Johnson (1925) rubbed tobacco leaves with sap from apparently healthy potato plants and chlorotic spots developed indicative of a mechanically transmitted virus. Yield reductions attributable to this disease are estimated as high as 75 per cent depending on virus strain, potato variety, and environmental conditions (Bawden et al., 1948; Clinch and McKay, 1949; Lombard, 1950; Norris, 1953a; Schultz and Bonde, 1944). Leaf symptoms on potato, influenced also by these factors, vary from complete masking to severe mottling or necrosis (Figures 1, 2).

Virus X disease is controlled mainly through eradication from partially infected stocks and by use of immune varieties. Older potato varieties such as Irish Cobbler, Russet Burbank, and Green Mountain, however, were universally infected with virus X before suitable means for detection were available. Since many of these varieties continue to be grown widely, a method for elimination of virus X from infected plants is desirable. A virus X-free clone of Green Mountain was obtained by treating meristem cultures with malachite green (Norris, 1954). The present study was undertaken to investigate the effectiveness and practical aspects of Norris' method and to develop a virus X-free clone of the Irish Cobbler potato variety.

Figure 1. Symptoms of virus X disease in potato leaves showing: (1) complete masking, (2) mild mottle, (3) severe mottle

Figure 2. Symptoms of virus X disease in potato leaflets showing: (1) complete masking, (2) mild mottle, (3) severe mottle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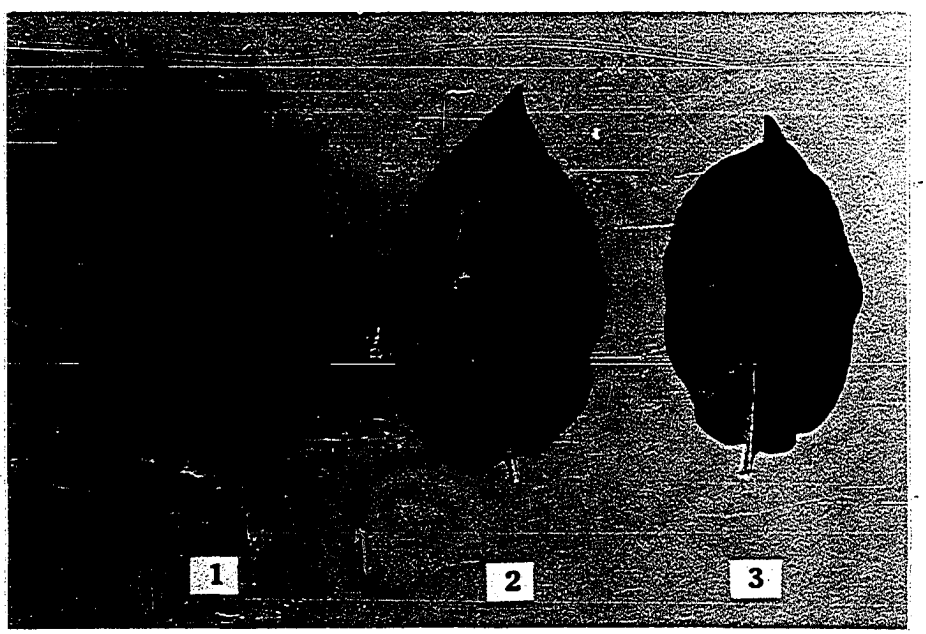
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REVIEW OF LITERATURE

Development of a virus X-free clone of the potato variety Green Mountain was first reported by Norris (1954). This clone was obtained by excision and culture of an apical meristem and treatment in culture with malachite green dye. Both meristem culture and malachite green treatment were considered necessary for elimination of virus X.

Meristem culture was used to obtain tissue with a relatively low virus titer. Limasset and co-workers (1949a, 1949b) showed meristematic tissue had a lower concentration of tobacco mosaic virus relative to other parts of the tobacco plant. Stapp and Bartels (1950) also found a decreasing concentration gradient of virus X from base to apex in potato tuber sprouts. Sheffield's data (1942) showed that tobacco mosaic virus was still present in very small sections of meristematic tissue. These reports suggested that virus-free tissue could not be obtained directly from meristem culture. Meristem culture could be used, however, if the virus remaining in the tissue could be eliminated.

Tobacco mosaic virus biosynthesis was shown by Takahashi (1948) to be inhibited by small amounts of malachite green dye. Norris (1954, p. 658) states, "Assuming that virus X would show similar behaviour, the maximum opportunity to obtain tissue free from virus would be to expose the extreme growing tips of potato to malachite green, and then excise

them to prevent re-invasion after treatment ceased". From 83 malachite green-treated cultures out of an original 200, Norris found one culture which proved to be virus X-free.

Many attempts have been made to confirm Norris' results. Thomson (1956b) was unsuccessful in all efforts to repeat Norris' work. He found, however, that subjecting sprout tips containing viruses X and Y to heat would eliminate virus Y (Thomson, 1956a, 1957). Morel and Martin (1955), on the other hand, reported consistent elimination of potato viruses X, Y and A using only meristem culture. Kassanis (1957b) recently reported finding four out of five untreated meristem cultures free of virus X. His cultures produced only callus growth, however, and only a small amount of tissue was thus available for testing. There have been many reports on the use of meristem culture for elimination of other plant virus diseases (Hildebrand, 1957; Holmes, 1955a, 1955b; Maramorosch, 1949; Quak, 1957). Apparently some virus diseases respond more favorably to this treatment than does virus X.

Successful elimination of virus diseases from infected plants has stimulated work on <u>in vivo</u> virus inactivation. Heat treatment has been one of the most successful older methods of plant therapy, but Kassanis' (1957a) recent review of heat treatment points out that <u>in vitro</u> temperatures of inactivation have no particular bearing on <u>in vivo</u> temperatures of inactivation. Thus heat treatment of plant material must be done empirically. Many chemicals also have been re-

ported effective in plant virus therapy. Stoddard (1947) found zinc sulfate, calcium chloride, quinhydrone, urea sodium thiosulfate, and other compounds would free bud wood of cherry X disease virus. Fulton (1954) reported *in vivo* inactivation of strawberry type two virus with zinc sulfate and calcium chloride, but was unable to show any antiviral effect of several antibiotics. Other workers have reported *in vivo* antiviral activity of materials such as gibberellic acid (Maramorosch, 1957), plant growth hormones (Kutsky and Rawlins, 1950), plant extractions (Allen and Kahn, 1957; Kassanis and Kleczkowski, 1948) and an antibiotic (Gray, 1955). Cytovirin, prepared by the Merck Chemical Company, is reported by Gray (1957), to be a very effective virus inhibitor but its extreme toxicity to man sharply limits its use[1]. Phytotoxicity is most often the factor which limits use of antiviral chemicals.

Malachite green appeared to have value as an antiviral agent because of its relatively low toxicity, but other workers have been unable to confirm its antiviral activity (Kirkpatrick and Lindner, 1954; Thomson, 1956b). Thomson suggested (1956b) that the apparent ineffectiveness of malachite green as a virus inhibitor may be due to a lack of tissue

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[1]Reed A. Gray, Microbiology Dept., Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey. Information on toxicity and use of cytovirin. Private communication. 1957.

penetration since Charles (1953) showed that basic dyes are much less readily absorbed by plant material than are acidic dyes. Takahashi (1957) recently repeated the work with malachite green and reported complete agreement with his earlier data. He states that the dye apparently inhibits only new virus synthesis and suggests a probable enzyme-blocking mode of action.

Among the more promising organic materials tested for *in vivo* antiviral properties have been purine and pyrimidine analogs (Kurtzman *et al.*, 1957; Matthews, 1954; Mercer *et al.*, 1953; Schlegel and Rawlins, 1954; Schneider, 1954). Thiouracil, a sulfur analog of the pyrimidine, uracil, has shown outstanding effectiveness as an inhibitor of viruses such as tobacco mosaic (Commoner and Mercer, 1952; Holmes, 1955c; Kirkpatrick and Lindner, 1954; Matthews, 1956; Nichols, 1953, 1954), cucumber mosaic (Porter and Weinstein, 1957), a stone fruit virus (Kirkpatrick and Lindner, 1954), and potato virus X (Bawden and Kassanis, 1954). Studies indicate that the antiviral effects of thiouracil can be reversed by addition of excess uracil, suggesting a nondisruptive, assimilative usage of the analog. Radiotracer investigations (Jeener and Rosseels, 1953; Mandel *et al.*, 1957; Matthews, 1956; Porter and Weinstein, 1957) have shown a replacement of uracil by thiouracil in the nucleic acid component of virus. Other compounds in this group appear to have a similar mode of action (Matthews, 1953).

Kirkpatrick and Lindner (1954) suggested that the apparent mild alteration of virus particles by thiouracil gives an inaccurate estimate of active virus titer when only physical measurements are employed. They found that spectrophotometry gave a higher virus titer when spectrophotometric and biological methods were used comparatively as assays for virus in thiouracil-treated plants. Steere (1955) reviews and discusses the problems of virus assay. He presents data comparing biological assay with measurement of non-biological properties using the electron microscope, spectrophotometer, electrophoresis, ultracentrifuge, serology and chemical analysis. Steere emphasizes the limitations of biological tests by pointing out 50,000 tobacco mosaic virus particles are apparently necessary to produce one local lesion. This discrepancy of many particles versus one infectious unit has obviously placed measurement of virus activity on a relatively uncertain basis, and yet infection and disease production are the ultimate tests.

MATERIALS AND METHODS

For preliminary studies begun at the University of Maine in 1954, details of Norris' meristem culture technique were not available. Sterilized excised potato tuber sprout tips were placed in 250 milliliter (ml.) erlenmeyer flasks containing 50 ml. of sterile White's (1954) liquid plant tissue culture medium. Liquid cultures were kept in darkness at 70° F., and aeration was accomplished by intermittent machine swirling. When growth was initiated, cultures were transferred to White's medium containing 0.7 per cent agar and placed in diffuse incandescent light at 70° F.

White's solution (1954), prepared as recommended, was used for most of the culturing (Table 1, column 1). This medium was also prepared using no correction for hydrates as suggested by Dr. A. E. Kehr (Table 1, column 2). Later in the study, errors in the formulation listed in White's book were pointed out by Dr. White[1], and a corrected medium was used (Table 1, column 3). Coconut milk and 2,4-D were added to White's solution in one experiment following the suggestion of Steward and Caplin (1951). In another experiment, White's solution was supplemented with water extract of autoclaved

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[1]P. R. White, Jackson Memorial Laboratory, Bar Harbor, Maine. Information on plant tissue culture media. Private communication. 1958.

tubers (Chapman, 1957). One-half strength Knop's solution (Morel and Martin, 1955) was used with White's vitamin formulation and two per cent sucrose. Some culturing was done using standard potato-dextrose-agar media. Analytical grade chemicals and pyrex glass-distilled or de-ionized water were used in preparation of all media. Media containing one part per million (ppm.) of naphthaleneacetic acid were used until growth was initiated since Skoog (1939) has shown any additional growth hormone after this time will inhibit growth.

New, wide-mouth 250 ml. pyrex erlenmeyer flasks containing 50 ml. of liquid or agar media were used. Beakers were used to cover the cotton plugs thereby reducing excessive moisture evaporation. Standard microscope slides wrapped with two thicknesses of nine centimeter (cm.) low ash filter paper, manufactured by W. and R. Balston, Ltd., England, acted as both a wick and a support for cultures in liquid (an adaptation of a method described by Norris) (Figure 5). Meristem cultures on agar were grown in eight-ounce glass bottles or petri plates (Figure 7). All glassware used for culture work and media preparation was thoroughly washed in hot one per cent "7X" solution, a washing compound made by The Limbro Chemical Company, Inc., New Haven, Conn., followed by several rinses in tap, distilled and double distilled water. After dispensing media were steam sterilized at 15 pounds for 15 minutes.

Table 1. Formulations of White's solution used for potato meristem culture

Chemical	Milligrams per liter[a]		
	1. Non-hydrate adjusted	2. Hydrate adjusted	3. Corrected hydrate adjusted
$MgSO_4 \cdot 7H_2O$	360.0	737.0	737.0
$Ca(NO_3)_2 \cdot 4H_2O$	200.0	287.9	287.9
Na_2SO_4	200.0	200.0	200.0
KNO_3	--	--	80.0
KCl	80.0	80.0	65.0
$NaH_2PO_4 \cdot H_2O$	16.5	19.0	19.0
$MnSO_4 \cdot 4H_2O$	4.5	6.7	6.7
$ZnSO_4 \cdot 7H_2O$	1.5	2.7	2.7
H_3BO_3	1.5	1.5	1.5
KI	0.75	0.75	0.75
Glycine	30.0	30.0	3.0
Nicotinic acid	5.0	5.0	0.5
Pyridoxine	1.0	1.0	0.1
Thiamine	1.0	1.0	0.1
Sucrose	20,000.0	20,000.0	20,000.0
$Fe_2(SO_4)_3 \cdot xH_2O$	2.5	2.5	2.5

[a]Columns 1 and 2 are taken from White (1954). Column 3 is corrected for typographical errors.

Foundation grade Irish Cobbler potatoes from Stark's Farms Inc. of Rhinelander, Wisconsin, and other varieties obtained from Dr. A. E. Kehr, Ames, Iowa, were used as meristem culture sources. Tuber dormancy was broken by treating with 2.2 ml. of 40 per cent ethylene chlorohydrin per kilogram of tubers for four days at room temperature. Sprout cuttings were taken from tubers sprouted on moist toweling in the dark at 60° F.

Sprout tips one to two millimeters (mm.) long were excised (Figure 4), dipped momentarily in 70 per cent ethanol, and placed for ten minutes in a 10 per cent volume for volume (v/v) solution of commercial Clorox. They were then moved to a holding solution of one per cent Clorox from which they were transferred to media (Figures 8, 9). Contamination by microorganisms was very low during initial transfer operations. A scalpel used for most cutting operations consisted of a broken piece of double-edged razor blade held in an X-acto knife handle (No. 1). The blade makes a very fine cut and can be discarded after use.

Cultures were also established by excision of meristematic tissue of young plants from either apical or lateral buds. Apical bud dissection and excision was done using the technique described by Norris (1954) (Figure 10). Lateral buds together with some axillary tissue were excised and placed on White's medium, either solid or on filter paper slopes. These

lateral buds very often developed into small tubers (Figure 11) which were excised and transferred to fresh media. Tubers formed in this way sprouted readily with no apparent dormancy (Figure 14).

Cultures usually produced shoots soon after roots were formed (Figures 12, 13) and these shoots were subdivided between nodes. Cutting was done under a flap of moist sterile paper toweling with either sterilized surgical scissors or a sterilized scalpel, and cuttings were transferred with a modified transfer loop to fresh media (Figures 15, 16, 17). Surface tension of liquid in the loop helped to hold the cuttings in place during manipulation. Culture flasks were held in place at a convenient angle during transfer operations with a holder shown in Figure 3.

Early culture work was done in a walkin refrigerator remodeled by Dr. A. E. Kehr for tissue culturing and located in the Horticulture Building at Iowa State College. This room, hereafter referred to as Kehr's culture room, was equipped with a gravity-flow refrigeration unit and an air filtering system. Walls, ceiling and work bench were panelled with asbestos board to facilitate cleaning with disinfectants. An ultraviolet sterilizing lamp and a glass transfer shield were installed as added deterents to culture contamination. Cultures were kept in this room in an aluminum alloy rack (Figure 6) in darkness at 70° F.;

lights were turned on only during the time when transfers or observations were made.

Later in the work, a walkin refrigerator in Botany Hall, Iowa State College, was equipped with a filtered air system, work bench, clear plastic transfer shield, ultraviolet lamp, and culture racks for use as a culture chamber. Light and temperature conditions in the chamber were similar to those previously described.

Autoclaved malachite green dye, made by The Matheson Chemical Company, was added aseptically to cultures at the rate of four and eight ppm. Sterile thiouracil, furnished by Dr. Paul F. Hoffman, Monsanto Chemical Company, was also added aseptically at rates five and ten ppm. The thiouracil solution was sterilized by passage through a bacterial filter rather than by heating since changes are sometimes induced by heat sterilization. Malachite green and thiouracil treatments were continued for one to three weeks. Approximately one-half of the cultures were so treated.

To prevent transplanting shock, it was necessary to precondition plantlets before transfer to soil by placing in covered glass dishes containing moist vermiculite and exposing to low intensity florescent lamps at room temperature. Covers were removed periodically to allow some exposure to the drier air, and finally were removed entirely. When plantlets became well-rooted and green, they were transferred to small


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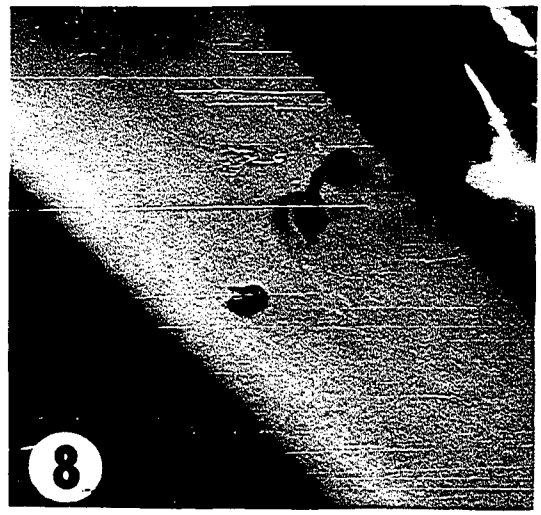
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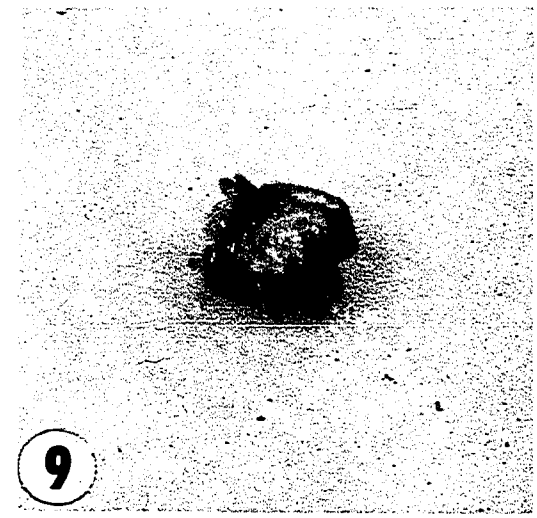
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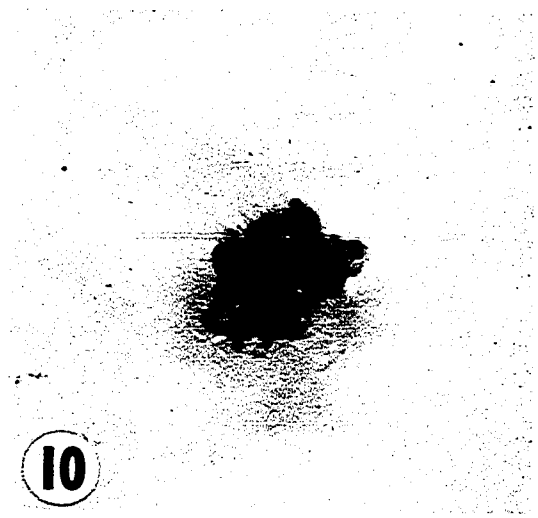
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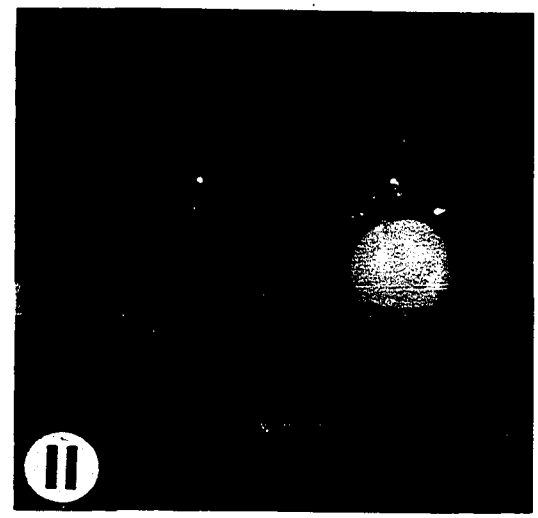
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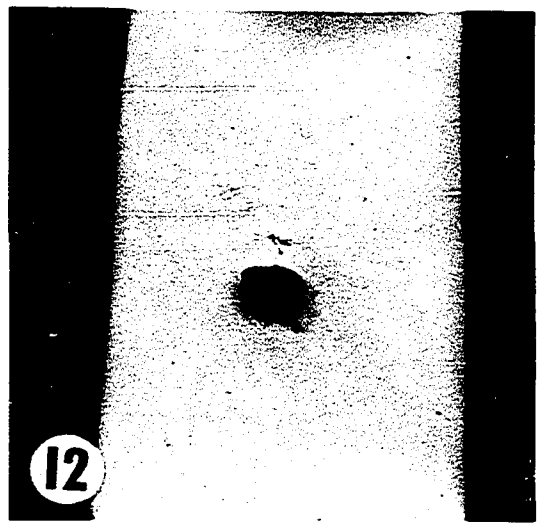
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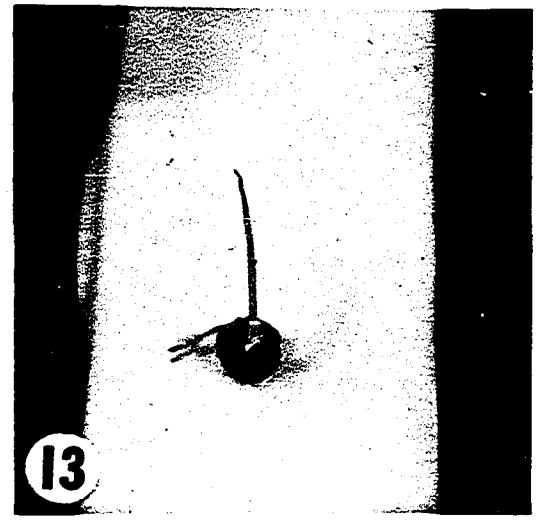
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Figure 14. Potato tuber formed from axillary bud with a sprout ready for subculturing

Figure 15. Subculture of potato meristem ready to be re-divided

Figure 16. Subculture of apical meristem of potato

Figure 17. Subculture of potato meristem from a node


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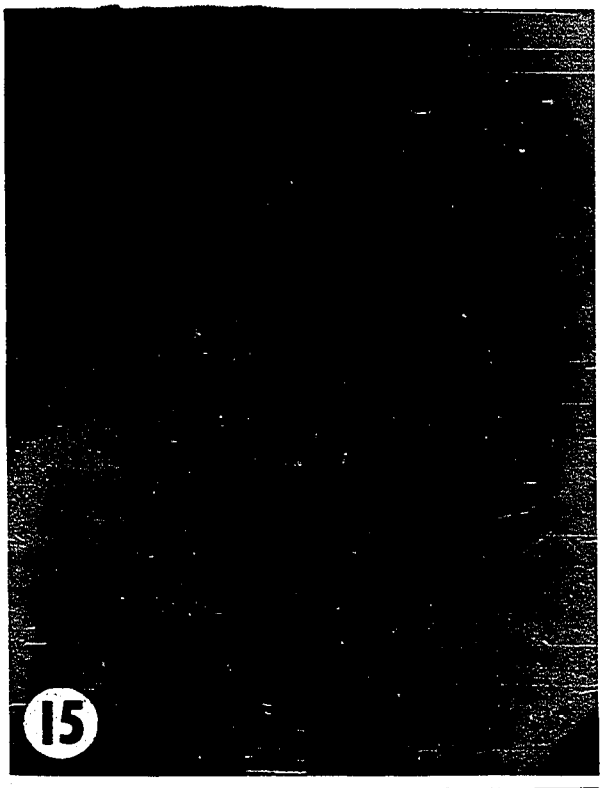
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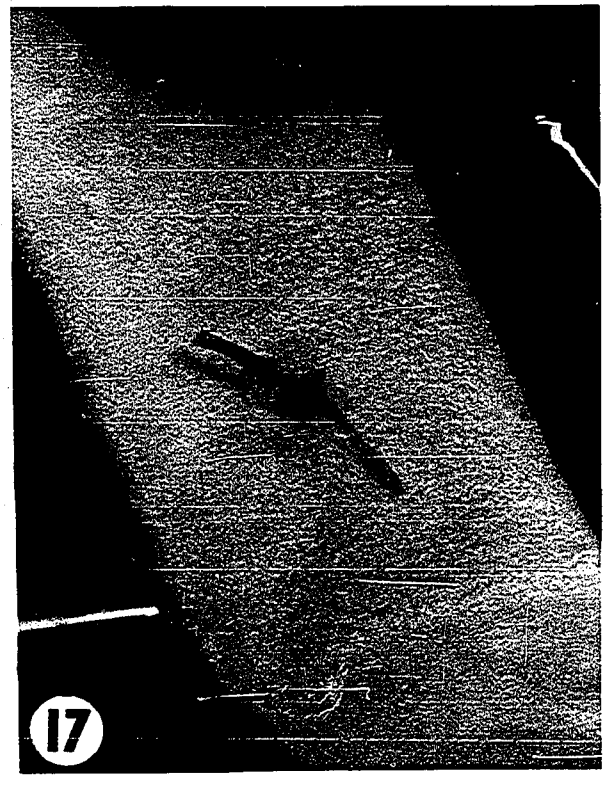
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pots of sterilized composted greenhouse soil (two parts loam, one part peat) and covered with beakers until they became established (Figures 20, 21). Plantlets were also placed in a glass case containing Perlite, a product of The Minnesota Perlite Corporation (Figures 18, 19), and handled in a similar manner after establishment. Perlite proved to be a much more satisfactory rooting medium than vermiculite. Light was maintained at a low level until the small plants had become established and had hardened sufficiently to be placed in direct sunlight.

Gomphrena globosa L., a local lesion host (Wilkinson and Blodgett, 1948), was used as an index plant for determinations of virus X (Figures 22, 23). Plants grown from seed were used originally, but slow germination and seedling growth of this plant made it desirable to find a more rapid method of propagation. Axillary shoot cuttings, treated with Rootone, a commercial rooting powder made by the American Chemical Paint Company, Ambler, Pennsylvania, were rooted in moist vermiculite, sand, or Perlite in a covered transparent case (Figure 24). When cuttings were well-rooted (three to four weeks) they were transplanted to soil where growth was fairly rapid at temperatures of 70° to 80° F. During winter months, supplemental light was used for 8 hours in each 24.

Prior to inoculation, test plants were dusted lightly with 600 mesh carborundum powder using a De Vilbiss powder

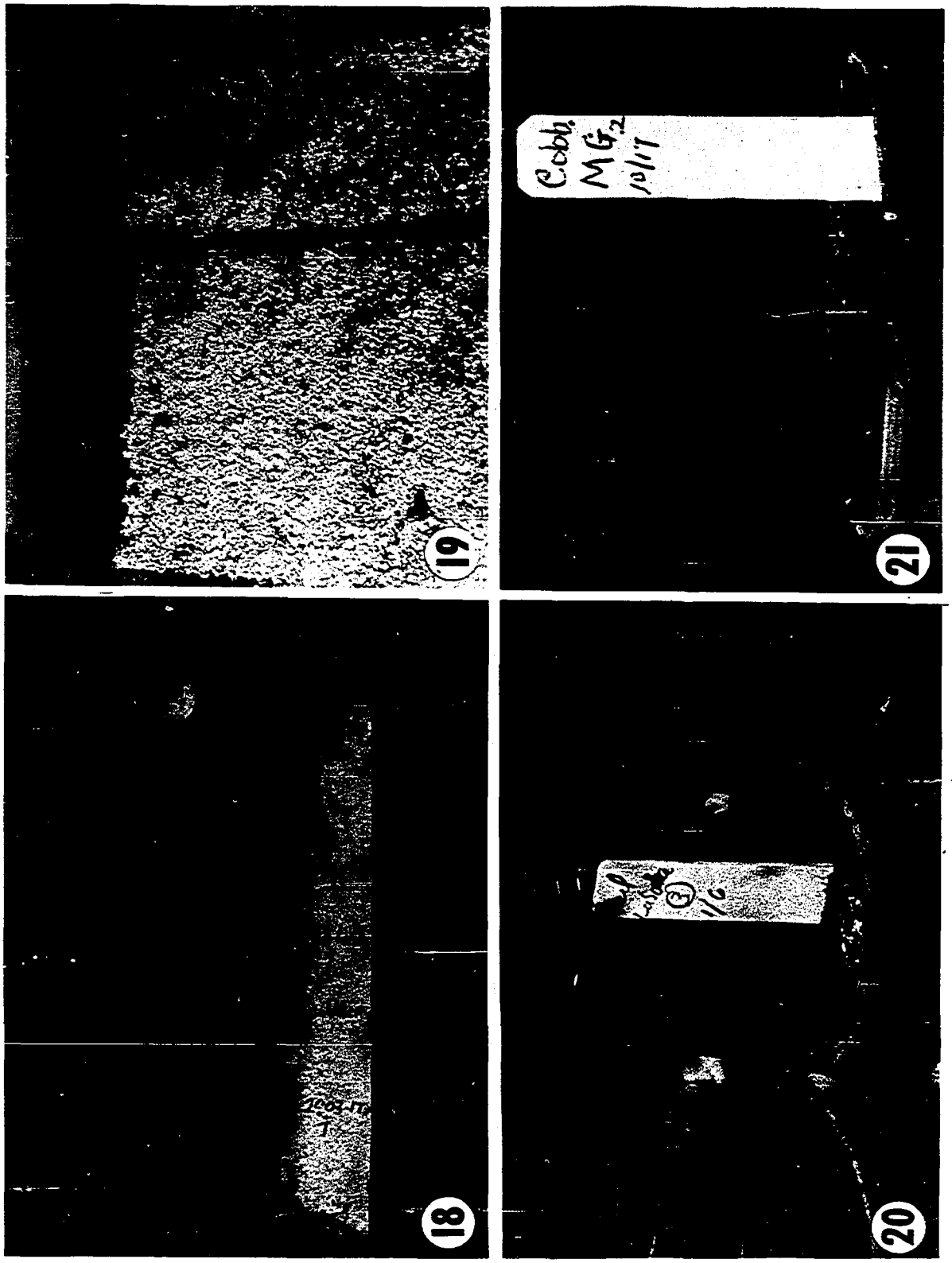
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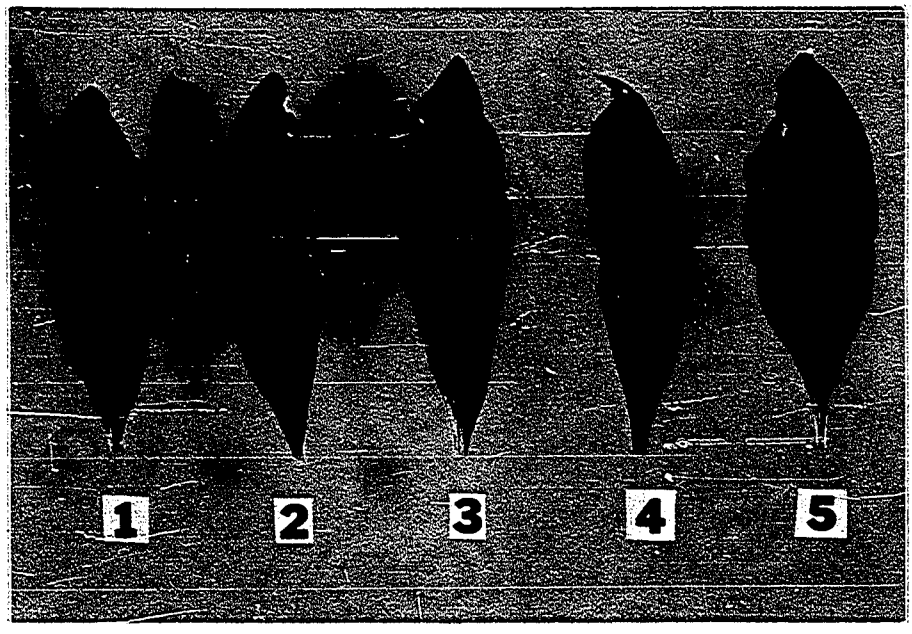
Figure 22. Virus X local lesions on upper leaf surfaces of Gomphrena globosa L. Leaves 1-5 are, respectively: healthy, four days, one week, two weeks and four weeks after inoculation

Figure 23. Virus X local lesions on lower leaf surfaces of Gomphrena globosa L. Leaves 1-5 are, respectively: healthy, four days, one week, two weeks and four weeks after inoculation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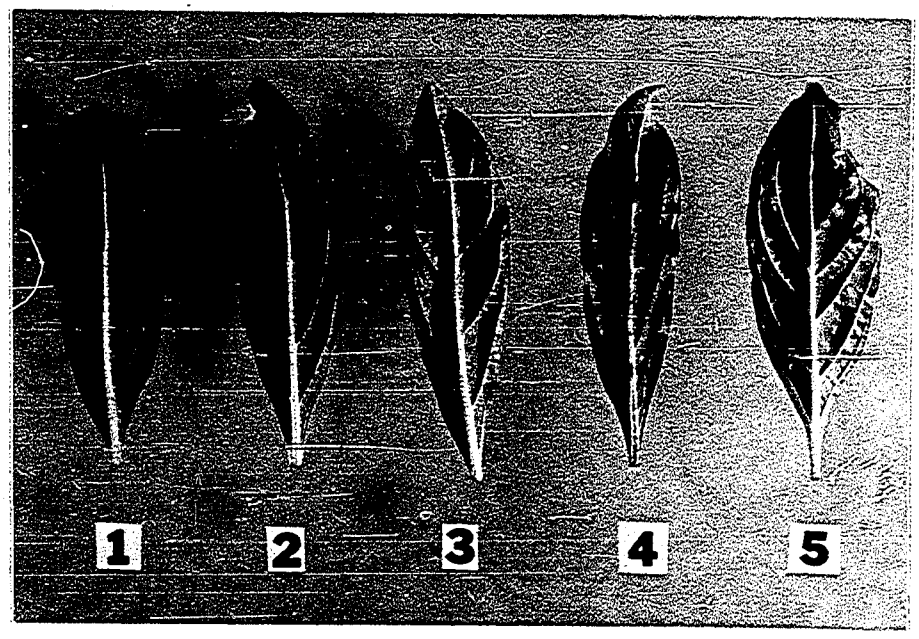
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blower. With sterile forceps, one or two leaves approximately one cm. in diameter from a plantlet to be tested were placed in a small plastic centrifuge tube containing two or three drops of .01 M sodium phosphate (PO_4) buffer of neutral pH. These leaves were macerated with a glass rod, which was also used for applying the macerate on test plant leaves labelled previously with small white tags (Figures 26, 27). Test tubes and glass rods were sterilized overnight in 10 per cent trisodium phosphate (Brock, 1952).

Foundation grade Irish Cobbler potatoes obtained from the Maine Seed Potato Board Farm, Masaris, Maine, and other varieties obtained from Dr. A. E. Kehr were treated with thiouracil in Experiments 1 to 4. Dormancy was broken using the ethylene chlorohydrin treatment previously described. All seedpieces were cut uniformly with a melon ball scoop and kept in groups for treatment with toothpicks or twine (Figure 28). Cut surfaces of seedpieces were not allowed to suberize before treatment. Thiouracil was applied by submerging seedpieces in water solution for various lengths of time at room temperature unless otherwise specified. A constant thiouracil rate of 100 ppm. was used for all experiments since Nichols (1953) showed thiouracil solutions of ten ppm. to 500 ppm. gave comparable results. Seedpieces used as controls were placed either in distilled water or in a moist chamber for 24 to 48 hours before planting. These experiments with

thiouracil were designed to determine its antiviral properties as affected by treatment time, solution temperature, amount of sprouting, location of sprouts and varietal differences. Seed-pieces were planted immediately after treatment in sterilized composted greenhouse soil in three-inch pots, and those seed-pieces which failed to produce plants were found to be decayed. Pots were spaced on the greenhouse bench to prevent possible mechanical transmission of virus X and plants which were found to be free of virus were placed in an isolated area (Figure 29).

Presence of active virus X was determined by assay on G. globosa unless otherwise indicated. A leaf to be tested was rolled and macerated using sterilized forceps and metal spatula. A small amount of buffer was added by dipping the forceps in PO_4 buffer prior to use. This sap-buffer mixture was smeared on a carborundum-dusted G. globosa leaf using the macerated potato leaf as a swab (Figure 25). Plants found free of virus X after G. globosa tests were also tested with the slide-agglutination reaction described by Bradley (1952). Virus X antiserum, prepared by Dr. E. L. Moorhead, Department of Plant Pathology, University of Nebraska, was diluted one to ten (1:10) with 0.9 per cent saline solution. A drop of diluted serum was mixed with a drop of crude sap on a microscope slide. Control comparative tests for non-viral agglutination were made by mixing a drop of saline instead

Figure 24. Rooting of shoot cuttings of G. globosa for propagation

Figure 25. Application of inoculum on test plant (G. globosa) leaves using the partially macerated potato leaf to be tested as an applicator

Figure 26. Inoculation procedure used on G. globosa when inoculum source is limiting

Figure 27. Inoculated G. globosa test plants tagged for identification


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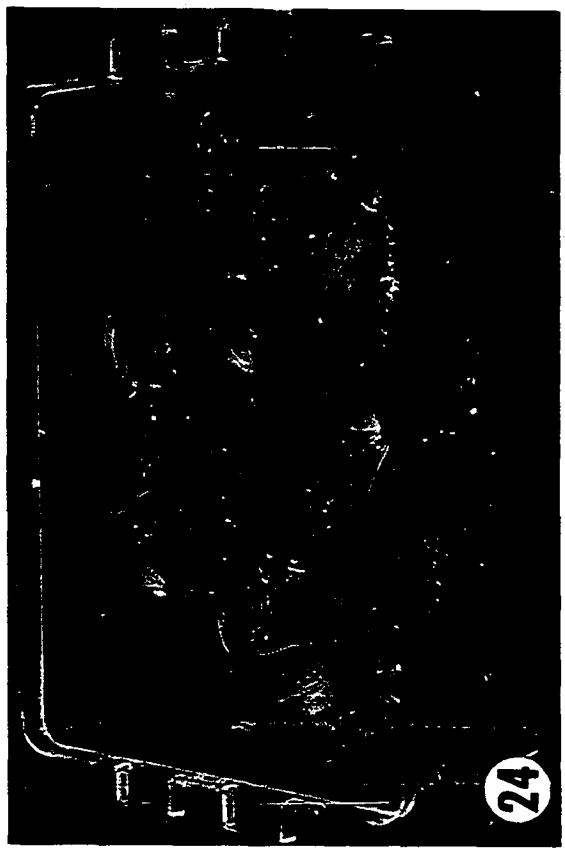
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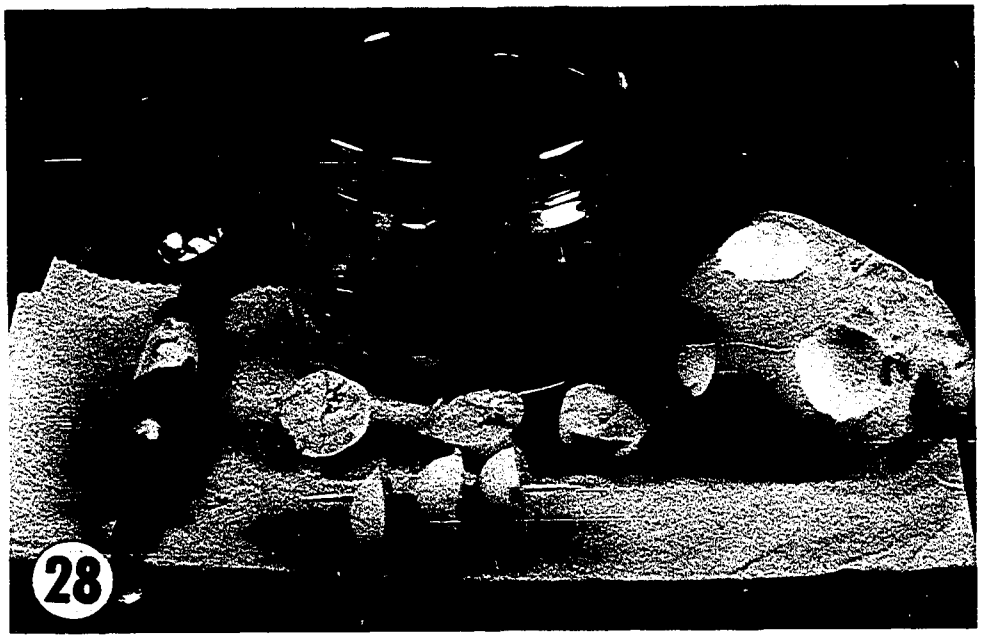
Figure 28. Techniques for cutting potato seed-pieces and treatment in thiouracil solution

Figure 29. Section of a greenhouse bench used as an isolation area for virus-free potato plants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of normal serum with a crop of crude sap on the other end of the slide. After three to five minutes, comparisons were made between the two drops and a positive test was indicated by flocculation of particles in the antiserum drop only.

Techniques for measuring physical properties of the virus were investigated as means of supplementing biological and serological assays. Etiolated Irish Cobbler and USDA seedling 41956 (virus X-immune) leaves were frozen, ground with a mortar and pestle, and the macerate expressed through four layers of cheesecloth. Crude sap from each source was centrifuged at 2000 G. and the supernatant was placed in a water bath at 50° C. for ten minutes before recentrifuging at 2000 G. Irish Cobbler preparations sampled for examination were crude sap, a 1:100 dilution of the supernatant from the first centrifugation, and a 1:10 dilution of the supernatant from heated recentrifuged sap. Seedling 41956 plant sap was treated in the manner described above, but a 1:10 dilution of heated recentrifuged preparation was the only sample examined. Three per cent "formvar" solution was used to prepare membranes which were mounted on standard 200 mesh specimen grids by the slide-film floatation method. Sap preparations were sprayed on prepared grids with a glass nebulizer, shadowed with gold, and examined with an RCA EMU electron microscope. Tests were made also on G. globosa of all sap preparations. Heated, recentrifuged Irish Cobbler

plant sap was deciserially diluted to 10^{-6} with distilled water. These dilutions were used to compare precision of the local lesion assay method with that of electron microscopy (Rochow et al., 1955). Another experiment was performed in which virus content of sap in various stages of sap clarification was assayed on G. globosa using deciseriate dilution with PO_4 buffer. Samples of virus X-infected and immune plant sap, prepared following standard methods of ultracentrifuge purification, were comparatively examined with a spectrophotometer. These samples also were assayed on G. globosa.

EXPERIMENTAL RESULTS

Potato Meristem Culture Work at the University of Maine

A summary report by Norris (1953b) suggested elimination of virus X from infected potatoes by meristem culture. Preliminary studies on meristem culture were begun at the University of Maine using the swirling culture method previously described. Three out of approximately 200 sprout cuttings produced shoot and root growth. These cultures were lost when the work was transferred to Iowa State College. Details of Norris' culture technique previously unavailable, were known when culturing was begun at Iowa State College, and the swirling method was discarded.

Potato Meristem Culture Work at Iowa State College

In November of 1955, 75 tuber sprout tip cuttings were started on slopes in Kehr's culture room. Only limited callus growth was produced until three months later when two cultures started root and shoot growth. A few of the remaining cuttings produced weak shoot and root growth after six to eight months, but most of these were subsequently lost through contamination by microorganisms. Root growth always preceded shoot growth and was initiated either in callus tissue or from the

parent cutting. When shoots were five to ten cm. long, they were subdivided between nodes and placed on fresh nutrient where new bud growth was initiated in some subcultures within one week. Growth of other subcultures, however, was either very slow or absent. These growth inconsistencies were unexplainable.

Early in 1956, 25 cuttings made from shoot tips of young plants were started on slopes. These cultures rarely produced root growth, and no shoot growth suitable for subculturing was ever obtained. Cultures derived from axillary buds, however, usually produced small tubers which sprouted readily and were excellent sources of shoots for subculturing.

Approximately 60 cultures obtained both from tuber sprout tips and axillary buds were available for subculturing by spring of 1956. Most of these cultures were lost at this time, however, due to contamination by microorganisms. Increased use of the culture room by others had caused wide temperature fluctuations resulting in moisture condensation inside the beakers covering the culture flask plugs. Before this condition was detected, the plugs became saturated with moisture and fungi grew through them showering spores on the culture medium. Only a few cultures could be saved and these were taken to the Botany Hall culture room where work was resumed.

When culturing was started in this new location, growth was found to be slow and abnormal. Transfer of cultures to

freshly prepared media did not produce normal growth. Fresh stock solutions were prepared to obviate possible alteration of original stocks, but normal growth still did not resume. Comparisons were made between new and old inorganic salt stock solutions for total dissolved phosphate, using an analysis described by Boltz and Mellon (1948), but no differences were indicated. These fresh stock solutions were considered to be equivalent to those previously used and attention was directed toward the culture room in an attempt to locate growth inhibiting factors.

A filtered forced air system, installed in the chamber, did not overcome the growth difficulty. Ultraviolet light in the chamber was omitted because Popp and McIlvaine (1937) showed it inhibited synthesis of growth substances, but resumption of growth still did not occur. A large sheet of transparent, quarter-inch Rohm and Haas plastic used as a transfer shield was removed from the culture room following the suggestion it contained volatile plasticizers inhibitory to plant growth. Cultures were then transferred to media which had not been stored in the culture room with the plastic, and growth was improved. Growth rates were, however, still less than previously observed.

Kehr's culture room was again vacant in the fall of 1957 and work was relocated there. Only two of the cultures brought back from the Botany Hall culture room resumed normal growth; these were increased by subculturing. Meristem

cultures of Red LaSoda and Russet Burbank which had been started by Dr. A. E. Kehr were also subcultured. These two potato varieties are known to be universally infected with virus X. Though culture growth was obtained in Kehr's culture room it was, as before, very erratic. Various culture media, used in an attempt to obtain more rapidly a sufficient number of cultures for replicated viricide treatments, gave comparable growth rates suggesting factors other than media were responsible. Culturing, therefore, was continued using White's solution (Table 1, column 2) and early in 1958 viricide treatments were begun.

Cultures treated with four ppm. of malachite green dye showed a reduced growth rate while those treated at eight ppm. made negligible growth (Table 2). Some fungus-contaminated cultures were treated with malachite green and fungus growth was effectively arrested at the eight ppm. rate. Thiouracil was found to be extremely phytotoxic and no cultures survived either treatment rate. Most malachite green-treated cultures were successfully recovered after treatment but many cultures were lost during transfer to soil. Positive virus X tests were shown on all plants derived from meristem cultures. Evidence based on comparisons of numbers of lesions on test plants indicated no reduction in virus titer due to malachite green treatment.

Table 2. Survival of potato plantlets from meristem cultures following treatment and transfer to soil

Variety	Treatment	Rate in ppm.	Number treated	Number surviving[a]
Irish Cobbler	Malachite green dye	4	20	3
"	"	8	20	4
"	Thiouracil	5	10	0
"	"	10	10	0
"	Untreated	--	20	2
Red LaSoda	Malachite green dye	4	15	2
"	"	8	15	3
"	Untreated	--	20	5
Russet Burbank	Untreated	--	3	1
Totals			133	20

[a]All survivors gave positive virus X tests on G. globosa.

Thiouracil Seedpiece Treatments

Treatment of potato seedpieces directly with a viricide such as thiouracil was suggested by Dr. Wm. G. Hoyman[1] as

[1]Wm. G. Hoyman, Plant Pathology Dept., Washington State University. Information on thiouracil treatment of potatoes for virus X elimination. Private communication. 1957.

an alternative to meristem culture for virus X elimination. Experiment 1 investigated the possibility of eliminating virus X by treatment of seedpieces in a solution of 100 ppm. thiouracil in water for various lengths of time at 70° F. Seedpieces from Irish Cobbler potatoes were treated in groups according to apical or basal location of tuber buds following Prochal's suggestion (1953) of possible differences in virus concentration between apex and base. Twenty plants from the 9- and 27-hour thiouracil treatments gave negative virus X tests two or three times, but Table 3 shows that only a total of nine plants remained virus X-negative after four tests at ten-day intervals. Treatment durations of 9 and 27 hours resulted in more seedpiece decay but a greater number of virus X-negative plants. Apical and basal buds showed no consistent differences in reaction to thiouracil or distilled water. Some seedpiece decay resulted from distilled water treatments but no virus X-negative plants were obtained. Five of the nine virus X-negative plants were lost from an over-application of fertilizer, but the remaining four have given negative tests in nine trials at ten-day intervals. They have also given negative serological tests for virus X. Results of Experiment 1 indicated thiouracil seedpiece treatments merited further study.

In Experiment 2, seedpieces of four virus X-infected potato varieties in addition to Irish Cobbler were used to

Table 3. Reaction of apical and basal Irish Cobbler seedpieces to thiouracil treatment for varying lengths of time. Experiment 1

Source of seedpieces	Duration of treatment (hours)			
	1	3	9	27
	Thiouracil[a]			
Apical buds	1/4/0	1/4/0	2/3/0	1/2/2
Basal buds	1/4/0	0/5/0	1/3/1	2/2/1
Totals	2/8/0	1/9/0	3/6/1	3/4/3
	Distilled water[a]			
Apical buds	0/5/0	0/5/0	0/4/1	0/4/1
Basal buds	0/5/0	0/5/0	0/5/0	0/3/2
Totals	0/10/0	0/10/0	0/9/1	0/7/3

[a]Virus X-negative / Virus X-positive / Decayed.

compare varietal responses to thiouracil. All seedpieces were treated for 24 hours since the longer thiouracil treatments in Experiment 1 gave more virus X-negative plants. Because no differential reaction was noted between apical and basal tuber buds in Experiment 1, seedpieces were selected at random from tubers. In addition to distilled water treatment, seedpieces were held in a moist chamber for 24 hours as a check on amount of seedpiece decay resulting from distilled water treatment. No virus X-negative plants resulted from

treatment and a very high incidence of seedpiece decay developed from both thiouracil and distilled water treatments (Table 4). One untreated Red Pontiac potato plant was found which gave a negative virus X test. This plant gave three additional negative tests in trials at ten-day intervals and also showed a negative serological test for virus X. Virus X-free Red Pontiac potato plants have been found occasionally by Hoyman[1] in routine indexing work, therefore, the plant found here was of no particular significance to the experiment.

Red LaSoda and Red Pontiac both had a higher incidence of seedpiece decay than the other varieties (Table 4). Seedpieces of these two varieties when treated had longer sprouts than the others. This suggested some relationship between sprouting and decay and appeared worthy of investigation.

Effects of 4- to 24-hour thiouracil treatments on long- and short-sprouted Irish Cobbler seedpieces were studied in Experiment 3. Check seedpieces were placed in thiouracil solution and distilled water for 24 hours but not submerged so effects of thiouracil on seedpiece decay could be measured in the absence of possible oxygen starvation effects. Seedpiece decay did not occur in distilled water treatments

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[1]Wm. G. Hoyman, Plant Pathology Dept., Washington State University. Results of greenhouse-indexing of potatoes for virus X. Private communication. 1957.

Table 4. Reaction of seedpieces of five potato varieties to thiouracil treatment for 24 hours. Experiment 2

Treatment	Variety				
	Garnet Chili	Irish Cobbler	Red LaSoda	Red Pontiac	Russet Burbank
Thiouracil[a]	0/6/9	0/6/9	0/2/13	0/0/15	0/7/8
Distilled water	0/4/11	0/5/10	0/3/12	0/4/11	0/11/4
Untreated[b]	0/10/5	0/12/3	0/15/0	1/14/0	0/14/1

[a]Virus X-negative / Virus X-positive / Decayed.

[b]Seedpieces cut and held in a moist chamber for 24 hours before planting.

but was high in thiouracil checks and eight-hour and longer thiouracil treatments, indicating thiouracil treatment resulted in seedpiece decay. Seedpiece decay was not associated with length of sprout. All plants in Experiment 3 were virus X-positive (Table 5).

In Experiments 2 and 3, thiouracil treatment resulted in seedpiece decay but elimination of virus X, shown in Experiment 1, was not demonstrated. In Experiment 1, conducted in the fall when only dormant tubers were available, tubers were treated with ethylene chlorohydrin to break dormancy. Thus, either tuber dormancy, or dormancy-breaking treatment or their combined effect could have contributed to successful

Table 5. Reaction of long- and short-sprouted Irish Cobbler seedpieces to thiouracil treatment for varying lengths of time. Experiment 3

Length of sprout	Duration of treatment (hours)				
	Check[a]	4	8	16	24
		Thiouracil[b]			
1 cm.	0/3/2	0/5/0	0/3/2	0/2/3	0/3/2
1 mm.	0/2/3	0/5/0	0/2/3	0/4/1	0/1/4
Totals	0/5/5	0/10/0	0/5/5	0/6/4	0/4/6
		Distilled water[b]			
1 cm.	0/5/0	0/5/0	0/5/0	0/5/0	0/5/0
1 mm.	0/5/0	0/5/0	0/5/0	0/5/0	0/5/0
Totals	0/10/0	0/10/0	0/10/0	0/10/0	0/10/0

[a]Seedpieces placed in liquid for 24 hours but not submerged.

[b]Virus X-negative / Virus X-positive / Decayed.

virus X elimination. Since dormant tubers were not available for additional experiments and treatment of non-dormant tubers with ethylene chlorohydrin causes tuber breakdown, these possible explanations could not be investigated.

An attempt was made, however, to substitute low temperature for tuber dormancy (Experiment 4). Seedpieces from sprouted and unsprouted Irish Cobbler tubers stored at 33° F. were treated at this temperature and at 70° F. in thiouracil

and distilled water for 12, 24 and 48 hours. Sprouted and unsprouted seedpieces used as controls were stored in moist chambers at 33° F. and 70° F. for 48 hours. Frequency of decayed seedpieces was very low in all thiouracil and distilled water treatments at 33° F. Thiouracil treatment at 70° F. for 24 hours resulted in decay of 30 per cent of the pieces whereas treatment in distilled water at the same temperature for an equal period gave only ten per cent decay indicating, as previously shown, that thiouracil treatment promotes seedpiece decay at the higher temperature. In 48-hour treatments at 70° F. thiouracil gave 75 per cent decay and distilled water gave 65 per cent suggesting that although decay occurred from both treatments thiouracil phytotoxicity resulted in greater injury. Only plants from thiouracil treatments were tested for virus X and all tests were positive (Table 6).

Other Virus X Tests

Although some thiouracil-treated plants which failed to give positive virus X tests on G. globosa were obtained in Experiment 1, the possibility existed that virus was present but in an undetectable form or quantity. Electron microscopy was used in an attempt to investigate this possibility. Results of a preliminary investigation (Figure 30), indicated heated, re-centrifuged Irish Cobbler plant sap gave preparations in which particle size and shape agreed with work of

Table 6. Reaction of sprouted and unsprouted Irish Cobbler seedpieces to thiouracil treatment at two temperatures for varying lengths of time. Experiment 4

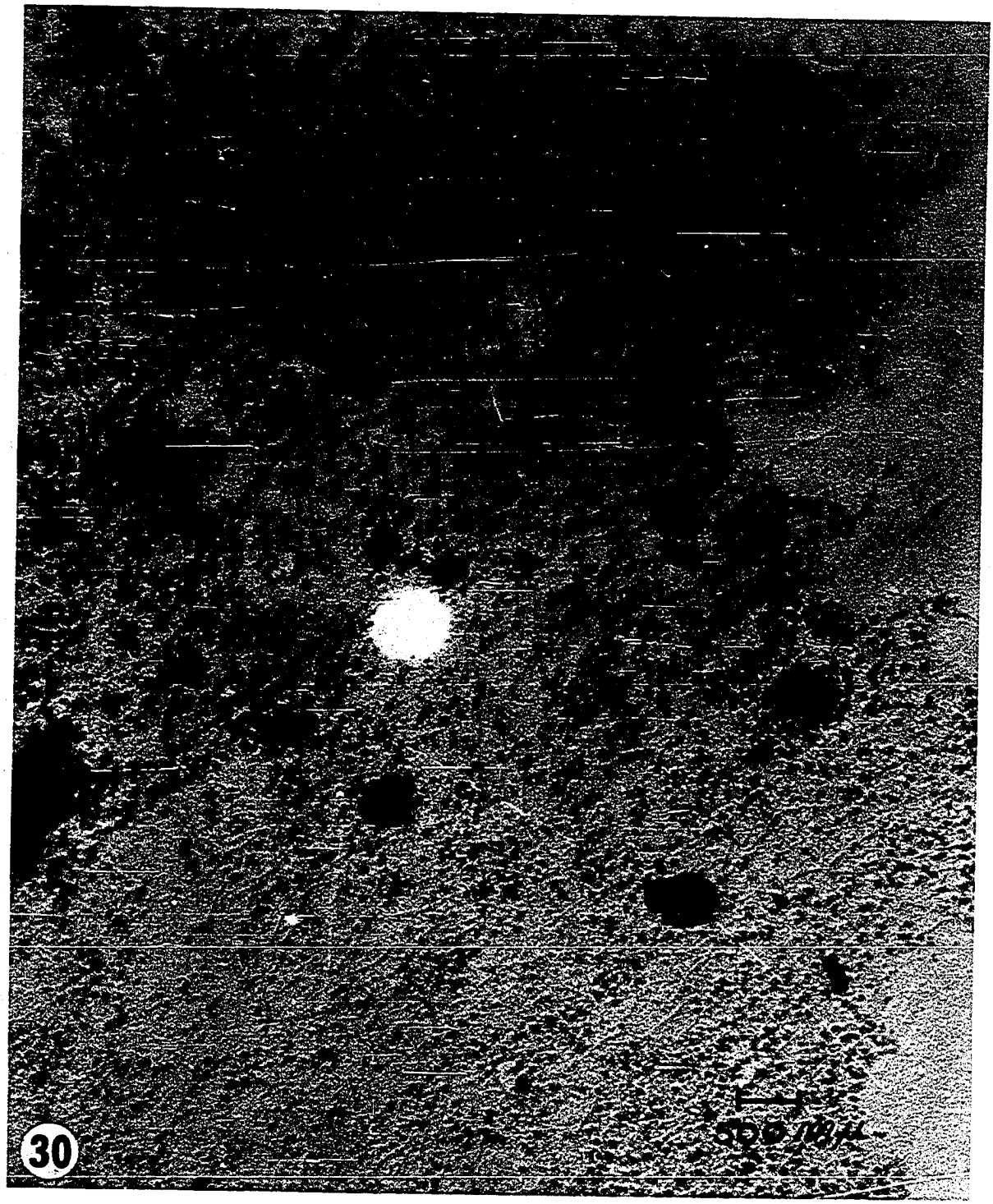
Seed-pieces	12 hours		24 hours		48 hours	
	33° F.	70° F.	33° F.	70° F.	33° F.	70° F.
			Thiouracil[a]			
Sprouted	0/9/1	0/10/0	0/10/0	0/7/3	0/9/1	0/2/8
Unsprouted	0/10/0	0/8/2	0/10/0	0/7/3	0/9/1	0/3/7
Totals	0/19/1	0/18/2	0/20/0	0/14/6	0/18/2	0/5/15
			Distilled water[b]			
Sprouted	-/-/0	-/-/0	-/-/0	-/-/0	-/-/0	-/-/7
Unsprouted	-/-/0	-/-/3	-/-/0	-/-/2	-/-/1	-/-/6
Totals	-/-/0	-/-/3	-/-/0	-/-/2	-/-/1	-/-/13
			Untreated[c]			
Sprouted					-/-/0	-/-/0
Unsprouted					-/-/0	-/-/0
Totals					-/-/0	-/-/0

[a]Virus X-negative / Virus X-positive / Decayed.

[b]No virus X tests were made-/ - / Seedpieces decayed out of ten.

[c]Ten seedpieces in each group were cut and held in a moist chamber for 48 hours before planting. No virus X tests were made.

Figure 30. Electron photomicrograph of clarified sap from a virus X-infected potato plant showing rod-shaped virus X particles. (approximately 18,000X)


30

Bode and Paul (1955). Dilution endpoint trials were performed on sap prepared in this manner to establish quantitative estimates of virus titer. Electron photomicrographs from each dilution were compared with G. globosa tests of each dilution. Positive tests were obtained on the test plant at a dilution of 1:1000, but the photomicrographs showed no recognizable particles at any of the dilutions. These results indicated that assays using electron microscopy must be replicated many times for validity, and such replication requires large amounts of time and plant material, both of which were limited in the present study. Spectrophotometric analyses of sap prepared using the ultracentrifuge were also inconsistent with G. globosa tests, therefore, G. globosa assay was selected as the determinative test for virus X.

In the previously described dilution endpoint trial on G. globosa, sap dilutions were made with distilled water because PO_4 buffer solution could not be used in preparations for electron microscopy. Since use of PO_4 buffer has been shown to increase sensitivity of local lesion test (Yarwood, 1957; Kahn and Libby, 1958) dilution endpoint trials were repeated using buffer for dilutions. Samples tested form all stages in sap clarification gave positive tests on G. globosa at dilutions of 1:1000, and crude sap and the supernatant from heated recentrifuged sap gave positive tests at 1:10,000 indicating that clarification procedures produced no change in virus concentration. These observations and

those of the previous dilution end-point trial showed that virus X could be reliably detected by G. globosa tests at a virus titer at least 1000 times lower than the titer routinely obtained from crude sap of Irish Cobbler potato plants.

One plant from Experiment 1 which tested negative for virus X was inoculated with sap from a normal (virus X-infected) Irish Cobbler plant. All inoculated leaflets of the thiouracil-treated plant died from inoculation injury before systemic infection could occur as evidenced by two negative tests for the virus. This plant was re-inoculated with a severe strain of virus X from Nicotiana glutinosa L. and symptoms were observed in three weeks on the inoculated leaflets. Positive virus X tests on G. globosa were obtained indicating that although virus X was apparently eliminated following thiouracil treatment, it could be re-introduced.

DISCUSSION

Inconsistencies of culture growth encountered in this study cannot be explained. Although conditions appeared to favor growth in Kehr's culture room, optimum results were not always obtained. Careful attention to water source, glassware cleanliness, media preparation, light and temperature did not insure good growth. Only empirical growth observations could be made following technique modification because of an apparent lag period following any change.

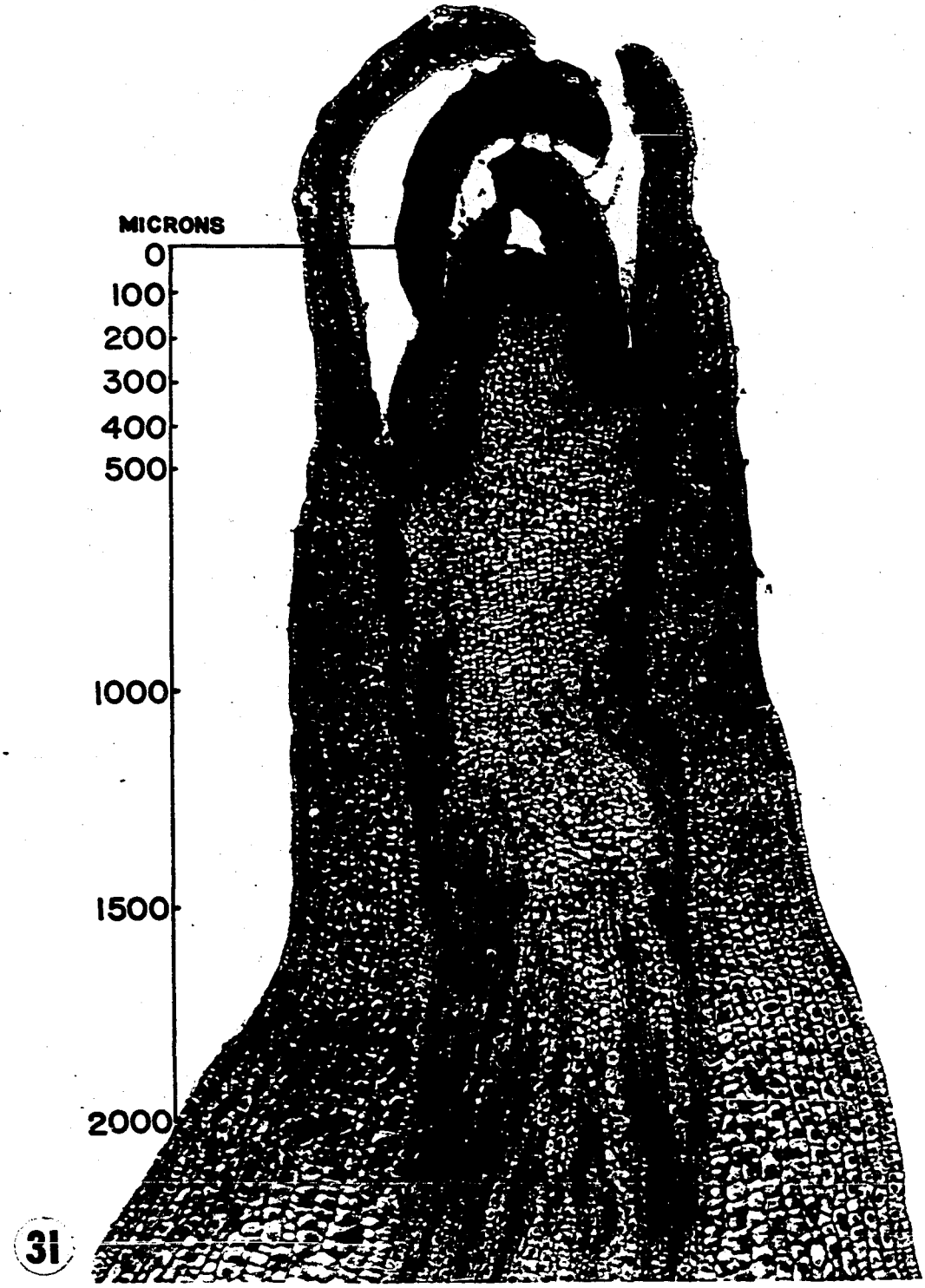
Normal culture growth was never achieved in the Botany Hall culture room despite all modifications. Removal of the plastic sheet, however, appeared to improve growth. Two young tomato plants held in the room for ten days showed symptoms similar to herbicide injury. Herbicides and fungicides are stored near the room and this may be a partial explanation for culture inhibition. Relocation of work to Kehr's culture room eliminated this problem and attempts to explain culture growth inhibition in the Botany Hall room were discontinued.

Kassanis (1957b) reported shoot and root growth in Morel and Martin's medium but only callus growth in White's medium. In the present study, both media supported good shoot and root growth, although abundant callus growth was common on White's medium. All variations in formulation of White's

solution and the various supplements used with this medium gave inconsistent results. Culture growth on other media was equally erratic indicating factors other than the medium were involved.

Size of cutting may be the key to success in virus X elimination by meristem culture. Morel and Martin (1955) used 100 to 200 micron sprout apices, did not subculture, and reported routine success without antiviral chemical treatment. Kassanis (1957b) used 100 to 250 micron apices and found the smaller sized cuttings gave negative virus X tests. Norris (1954) made an original cutting of 200 microns, treated resultant subcultures with malachite green, and then made two to five mm. cuttings from tips of these subcultures. Only one cutting from a treated culture proved to be virus X-free indicating that the original 200 micron cutting contained virus. Thomson (1956b) used five mm. apices which were not subcultured and of 154 malachite green-treated cultures tested, none was virus-free. In the present study all cuttings, transfers or re-isolations were two to five mm. long and no virus-free material was obtained. Sizes of meristem apices discussed above are shown in Figure 31. Norris (1954) used malachite green effectively, but Thomson (1956b) and the author were unable to demonstrate any viricidal effect of the dye. Use of 100 micron apical meristem cuttings appears to give consistently virus-free material but use of larger

Figure 31. Potato tuber sprout tip (approximately 75x)


MICRONS
0
100
200
300
400
500
1000
1500
2000
31

cuttings with or without viricide apparently is successful only rarely.

Achievement of satisfactory culture growth is essential to virus X elimination by meristem culture, and unknown growth-inhibiting factors can greatly hamper culturing. Loss of cultures from contamination by microorganisms and during transfer of cultures to soil further aggravates the problem. For these reasons meristem culture for virus X elimination appears to be of limited practical value. However, in certain virus diseases where extremely small cuttings are not necessary or where culturing can be simplified, this technique has been and undoubtedly will continue to be of considerable value.

Virus X elimination through chemotherapy was investigated as an alternative to meristem culture. Several workers have used thiouracil as a pre-infection treatment to inhibit biosynthesis of viruses, but use of this chemical as a chemotherapeutant for established plant-virus infection has been reported only by Holmes (1955c). In the present study, four thiouracil-treated Irish Cobbler potato plants gave consecutively nine negative virus X tests on G. globosa. These results were unconfirmed in subsequent experiments suggesting a synergistic effect of thiouracil with either or both of two factors: First, since virus X titer has been shown by Prochal (1953) to be lowest in dormant tissue, dormant tubers may have contributed to successful virus X

elimination in Experiment 1; secondly, ethylene chlorohydrin, used to break dormancy, may have influenced results either alone or in combination with tuber dormancy.

Complete virus elimination in thiouracil-treated plants reported in this study has not been established, but G. globosa tests showed these plants contained less than 0.1 per cent of the virus X titer found in healthy-appearing Irish Cobbler potato plants. The replacement of uracil by thiouracil in virus nucleic acid has been shown to reduce virus biological activity, and for this reason, thiouracil-treated plants could contain biologically active virus X in a titer too low to be detected by G. globosa tests. If virus X has not been completely eliminated in the treated plants, its titer may increase since it has been shown that virus inhibition by thiouracil is reversed by excess uracil. Virus X was detected only after two or three tests at ten-day intervals in some treated plants indicating that effects of thiouracil were slowly being reversed. This reversal of virus inhibition with time suggests that the plants which have given nine negative virus X tests may eventually give positive tests. One of these treated plants, however, was inoculated with virus X and infection was demonstrated suggesting this plant was free of the virus before this inoculation. Ease and effectiveness justify consideration of thiouracil treatment for routine virus X elimination.

Assay of virus X in thiouracil-treated plants by measurement of virus physical properties was attempted in order to establish presence of biologically inactive virus particles. Serological tests did not demonstrate presence of virus but there is no positive evidence to show that thiouracil does not alter virus antigenic properties. Use of electron microscopy was not pursued further because of limited plant material available and erratic results experienced. Irish Cobbler sap, prepared using the ultracentrifuge, was comparatively assayed for virus X with the spectrophotometer and on G. globosa. Results of the former assay were inconsistent with those of the latter. G. globosa assay, therefore, was selected as the determinative virus X test because it measured virus biological activity and gave consistent results.

SUMMARY

Meristem culture with or without malachite green dye treatment yielded no virus X-free potato plantlets or measurable reduction in virus titer. Parent meristem apices, subcuttings and re-isolations from treated cultures, two to five mm. long, were cultured on several types of liquid and solid media. Considerable difficulty was experienced in achieving and maintaining satisfactory culture growth. Use of meristem culture and malachite green treatment for virus X elimination appears to be extremely limited in practical application and effectiveness.

Thiouracil-treated Irish Cobbler seedpieces yielded four plants which over four months gave consecutively nine negative tests for virus X on Gomphrena globosa L. These plants also gave negative serological tests for virus X. Seedpieces were submerged in a 100 ppm. water solution of thiouracil for varying lengths of time. Treatment for as little as one hour resulted in one virus X-negative plant. Considerable seedpiece decay after planting occurred in pieces treated for 24 hours or longer. One experiment with dormant tubers yielded virus X-negative plants and it was suggested that dormant tubers or dormancy-breaking treatment contributed to the effectiveness of thiouracil treatment since in other experiments sprouting tubers were used and no virus

X-negative plants were obtained. Ease and effectiveness justify consideration of thiouracil treatment for routine virus X elimination.

LITERATURE CITED

Allen, Thomas C., Jr. and Robert P. Kahn. 1957. Tobacco mosaic virus inhibition by rice extracts. (Abstract) Phytopathology 47: 515.

Bawden, F. C. and B. Kassanis. 1954. Some effects of thiouracil on virus-infected plants. Jour. Gen. Microbiol. 10: 160-173.

_____, _____ and F.M. Roberts. 1948. Studies on the importance and control of potato virus X. Ann. Appl. Biol. 35: 250-265.

Bode, O. and H. L. Paul. 1955. Elektronenmikroskopische untersuchungen über kartoffel-viren. I. Vermessungen un Teilchen des Kartoffel-X-virus. (English summary.) Biochim. et Biophys. Acta 16: 343-345.

Boltz, D. F. and M. G. Mellon. 1948. Spectrophotometric determination of phosphorus as molybdiphosphoric acid. Analytical Chem. 20: 749-751.

Bradley, R. H. E. 1952. A rapid method of testing plants in the field for potato virus X. Amer. Potato Jour. 29: 289-291.

Brock, R. D. 1952. The use of trisodium phosphate as an inactivating agent for plant viruses. Jour. Aust. Inst. Agric. Sci. 18: 41-43.

Chapman, H. W. 1957. Continuous growth of excised potato root tips. Amer. Jour. Bot. 43: 468-471.

Charles, A. 1953. Uptake of dyes into cut leaves. Nature 171: 435-436.

Clinch, Phyllis E. M. and Robert McKay. 1949. A further experiment on the effect of mild strains of virus X on yield of up-to-date potato. Roy. Dublin Soc. Sci. Proc. 25: 93-99.

Commoner, B. and F. L. Mercer. 1952. The effect of thiouracil on the rate of tobacco mosaic virus biosynthesis. Arch. Biochem. and Biophys. 35: 278-289.

Fulton, R. H. 1954. Studies on inactivation of the strawberry type 2 virus *in vivo*. (Abstract) Phytopathology 44: 489.

Gray, R. A. 1955. Activity of an antiviral agent from *Nocardia* on two viruses in intact plants. Phytopathology 45: 281-285.

_____. 1957. Combating plant virus diseases with a new antiviral agent, Cytovirin. U.S. Dept. Agr. Plant Dis. Reptr. 41: 576-578.

Hildebrand, E. M. 1957. Freeing sweetpotato varieties from cork virus by propagation with tip cuttings. (Abstract) Phytopathology 47: 452.

Holmes, Francis O. 1955a. Elimination of a virus from sweet potato. Phytopathology 45: 185.

_____. 1955b. Elimination of spotted wilt from dahlias by propagation of tip cuttings. Phytopathology 45: 224-225.

_____. 1955c. Preventive and curative effects of thiouracil treatments in mosaic-hypersensitive tobacco. Virology 1:1-9.

Jeener, R. and J. Rosseels. 1953. Incorporation of 2-thiouracil-35S in the ribose nucleic acid of tobacco mosaic virus. Biochim. et Biophys. Acta 11: 438.

Johnson, J. 1925. Transmission of viruses from apparently healthy potatoes. Wis. Agr. Expt. Sta. Res. Bull. 63.

Kahn, Robert P. and John L. Libby. 1958. The effect of water and potassium phosphate buffer on plant virus infection prior to inoculation. Phytopathology 48: 57-60.

Kassanis, B. 1957a. Effects of changing temperature on plant virus diseases. Adv. Virus Res. 4: 222-241.

_____. 1957b. The use of tissue cultures to produce virus-free clones from infected potato varieties. Ann. Appl. Biol. 45: 422-427.

_____ and A. Kleczkowski. 1948. The isolation and some properties of a virus inhibiting protein from *Phytolacca esculenta*. Jour. Gen. Microbiol. 2: 143-153.

Kirkpatrick, H. C. and R. C. Lindner. 1954. Studies concerning chemotherapy of two plant viruses. Phytopathology 44: 529-533.

Kurtzman, Ralph H., Jr., Albert C. Hildebrandt, Robert H. Burris and A. J. Riker. 1957. Inhibition of tobacco mosaic virus multiplication and effects on host tobacco tissue cultures by substituted purines. (Abstract) Phytopathology 47: 527.

Kutsky, R. J. and T. E. Rawlins. 1950. Inhibition of virus multiplication by naphthalene acetic acid in tobacco tissue cultures as revealed by a spectrophotometric method. Jour. Bact. 60: 763-766.

Limasset, P. and P. Cornuet. 1949a. Recherche du virus de la mosaïque du tabac (Marmor tabaci Holmes) dans les meristèmes des plantes infectées. Comp. Rend. Acad. Sci. Paris 228: 1971-1972.

_____, _____ and Y. Gendron. 1949b. Titrage du virus de la mosaïque de tabac (Marmor tabaci Holmes) dans les organes aériens de tabacs infectées. Comp. Rend. Acad. Sci. Paris 228: 1888-1890.

Lombard, P. M. 1950. The effect of a mild strain of latent mosaic virus X on yield of some of the new potato varieties in Maine. Amer. Potato Jour. 27: 445-449.

Mandel, H. George, Roy Markham and R. E. F. Matthews. 1957. The distribution of thiouracil in nucleic acid of tobacco mosaic virus. Biochim. et Biophys. Acta 24: 205-206.

Maramorosch, K. 1949. Occurrence of virus-free cuttings from sweet-clover infected with wound-tumor virus. U.S. Dept. Agr. Plant Dis. Reptr. 33: 145.

_____. 1957. Reversal of virus-caused stunting by gibberellic acid. (Abstract) Phytopathology 47: 528.

Matthews, R. E. F. 1953. Incorporation of 8-azaguanine into nucleic acid tobacco mosaic virus. Nature 171: 1065.

_____. 1954. Effects of some purine analogues on tobacco mosaic virus. Jour. Gen. Microbiol. 10: 521-532.

_____. 1956. Thiouracil in tobacco mosaic virus. Biochim. et Biophys. Acta 19: 559.

Mercer, Frank L., Taylor E. Lindhorst and Barry Commoner. 1953. Inhibition of tobacco mosaic virus biosynthesis by 2-thiopyrimidines. Science 117: 558-559.

Morel, G. and C. Martin. 1955. Guérison de pommes de terre atteintes de maladies à virus. Comp. Rend. Acad. Agric. France 41: 472-475.

Nichols, C. W. 1953. Thiouracil inhibition of tobacco mosaic virus multiplication. Phytopathology 43: 555-557.

_____. 1954. Absorption of thiouracil by tobacco leaf tissue and inhibition of tobacco mosaic virus multiplication. Phytopathology 44: 92-93.

Norris, D. O. 1953a. The effect of virus X on yield of potatoes - an assessment. Jour. Aust. Inst Agric. Sci. 19:251-256.

_____. 1953b. Reconstitution of virus X-saturated potato varieties with malachite green. Nature 172: 816.

_____. 1954. Development of virus-free stock of Green Mountain potato by treatment with malachite green. Aust. Jour. Agric. Res. 5: 658-663.

Popp, H. W. and H. R. C. McIlvaine. 1937. Growth substances in relation to the mechanism of the action of radiation on plants. Jour. Agr. Res. 55: 931-936.

Porter, Clark A. and Leonard H. Weinstein. 1957. Biochemical changes induced by thiouracil in cucumber mosaic virus-infected tobacco plants. Contrib. Boyce Thompson Inst. 19: 87-106.

Prochal, P. 1953. Rozmieszczenie utajonego wirusa X w. bulwie Ziemniaczanej. (The distribution of the masked virus X in potato tubers.) Acta Agrobot. 1: 33-77. (Original not available for examination; abstracted in Rev. Appl. Mycol. 1954. 33: 441.)

Quak, F. 1957. Meristeemcultuur, gecombineerd met warmtebehandeling, voor het verkrijgen van virusurije anjerplanten. (Meristem culture, combined with heat treatment in order to obtain virus-free carnation plants.) Tijdsch. Plantenziekten 63: 13-14. (English summary)

Rochow, W. F., A. F. Ross and B. M. Siegel. 1955. Comparison of local-lesion and electron-microscope particle count methods for assay of potato virus X from plants doubly infected by potato viruses X and Y. Virology 1: 28-39.

Schlegel, David E. and T. E. Rawlins. 1954. A screening test of the effect of organic compounds on production of tobacco mosaic virus. Jour. Bacteriol. 67: 103-109.

Schneider, I. R. 1954. The effect of purines, purine analogs and related compounds on the multiplication of tobacco mosaic virus. Phytopathology 44: 243-247.

Schultz, E. S. and R. Bonde. 1944. The effects of latent mosaic (virus X) on yield of potatoes in Maine. Amer. Potato Jour. 21: 278-283.

Sheffield, F. M. L. 1942. Presence of virus in primordial meristems. Ann. Appl. Biol. 29: 16-17.

Skoog, Folke. 1939. Experiments on bud inhibition with indole-3-acetic acid. Amer. Jour. Bot. 26: 702-707.

Stapp, C. and R. Bartels. 1950. Der Serologische Nachweis des X-virus in Dunkelkeimen der Kartoffelknolle. Zuchter. 20: 42-47.

Steere, Russel L. 1955. Concepts and problems concerning the assay of plant viruses. Phytopathology 45: 196-208.

Steward, F. C. and S. M. Caplin. 1951. A tissue culture from potato tuber: the synergistic action of 2,4-D and coconut milk. Science 113: 518-520.

Stoddard, E. M. 1947. The X-disease of peach and its chemotherapy. Conn. Agric. Expt. Sta. Bull. 506: 1-19.

Takahashi, W. N. 1948. The inhibition of virus increase by malachite green. Science 107: 226.

_____. 1957. The inhibition of virus infection by malachite green. (Abstract) Phytopathology 47: 535.

Thomson, A. D. 1956a. Heat treatment and tissue culture as a means of freeing potatoes from virus Y. Nature 177: 709.

_____. 1956b. Studies on the effect of malachite green on potato viruses X and Y. Aust. Jour. Agr. Res. 7: 428-434.

_____. 1957. Elimination of potato virus Y from a potato variety. New Zealand Jour. Sci. and Technol. Ser. A. 38: 482-490.

White, P. R. 1954. The cultivation of animal and plant cells. New York, Ronald Press Co.

Wilkinson, R. E. and F. M. Blodgett. 1948. Gomphrena globosa; a useful plant for qualitative and quantitative work with potato virus X. (Abstract) Phytopathology 38: 28.

Yarwood, C. E. 1957. Mechanical transmission of plant viruses. Adv. Virus Res. 4: 243-278.

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